International Journal of Bio-Technology and Research (IJBTR)
ISSN(P): 2249–6858; ISSN(E): 2249–796X
Vol. 9, Issue 2, Dec 2019, 15–30

TRANS STELLAR
•Journal Publications •Research Consultancy

REMOVAL OF HEAVY METAL FROM INDUSTRIAL WASTE WATER USING BIOFILM PRODUCING BACTERIA

MADHUMALA Y[1], MANJULA MATHAPATI[2] & Dr. VEENA S. SORAGANVI[3]

[1]*Assistant Professor, Department of Biotechnology, Basaveshwar Engineering College, VTU, Bagalkot, Karnataka, India*

[2]*Department of Civil Engineering, Basaveshwar Engineering College, VTU, Bagalkot, Karnataka, India*

[3]*Professor, Department of Civil Engineering, Basaveshwar Engineering College, VTU, Bagalkot, Karnataka, India*

ABSTRACT

Heavy metals are a diverse group of compounds with varied characteristics. These metals are poisonous to plants, animals and humans, so they regarded a category of environmental pollutants. Hexavalent Chromium is one of the elements which is toxic and carcinogenic. Electroplating industry is one of the sources which produce waste water containing elevated concentration of heavy metals. Therefore, before discharging into the water body, wastewater should be treated.

In this study, the bisorption technique is used for the removal of hexavalent chromium from electroplating industrial wastewater, Belgaum. Two isolates Bacillus species (TF_1) and Staphylococcus aureus (G_5) are used as biosorbents. The design of experiments, i.e. Response surface Methodology (RSM) is carried out to optimize reduction process. For the reduction of Cr (VI) with concentration of 500mg/L, pH 6, glucose 0.6mg/l and biomass concentration of 6% was found to be the most ideal factors for both the isolates. Even enzymatic study is carried out to check the type of mechanism involved in the reduction and it was found that biosorption process involved here is an intracellular process. Both isolates in the industrial wastewater shows 100% reduction of Hexavalent Chromium in a period of 4 days and SEM analysis of Bacillus species was carried out.

KEYWORDS: Biosorption, Hexavalent Chromium, Bacillus Species & Biomass Concentration

Received: Jul 08, 2019; **Accepted:** Jul 30, 2019; **Published:** Nov 04, 2019; **Paper Id.:** IJBTRDEC20192

Original Article

1. INTRODUCTION

Water is essential for all life forms, but water pollution and destruction of ecosystem continue to increase day by day. Water contamination is now one of the major problems due to the consequence of industrialization, urbanization, population growth, globalization, etc. Industrial effluents which contain heavy metals may regard as a major source of contamination which are causing severe issues with the environment. Heavy metals are very diverse group of elements with differing chemical properties and biological functions. These are kept under environmental pollutants due to toxicity to plants, animals and humans. Heavy metals are 'Arsenic (As)', 'Cadmium (Cd)', 'Chromium (Cr)', 'Copper (Cu)', 'Lead (Pb)', 'Mercury (Hg)', 'Nickel (Ni)', 'Silver (Ag)' and 'Zinc (Zn)'.

Chromium is a chemical element which is hard, brittle metal having high melting point and a characteristic one. It appears in the form of Trivalent Chromium [Cr (III)] and hexavalent Chromium [(VI)]. Cr (VI) is toxic and carcinogenic. Deserted Chromium production facilities often need to be cleaned from the environment. Removal of heavy metals from industrial wastewater can be performed by multiple treatment techniques, such a 'chemical

precipitation', 'coagulation', 'complexation', 'activated carbon adsorption', 'ion exchange', etc. Precipitation is most commonly used among these techniques, but this creates incomplete processing and toxic sludge production. To develop cost-effective and more efficient metal adsorption methods, countless novel methods have been researched.

Biosorption: It is regarded as one of the user-friendly, efficient methods of purification and separation for removing of metals from the industrial wastewater with benefits of particular affinity, low price and easy design [1] [2]. It was therefore commonly used for treatment. The method of biosorption includes a solid phase (sorbent, biological material) and fluid phase (solvent, usually water) that contains dissolved species to be sorbed (sorbate, metal ions).

A biofilm is a cluster of microorganism where cells bind to each other and frequently adhere to a solid surface. In a self-produced matrix of extracellular polymeric substances (EPS), these adherent cells are often integrated. Biofilm organisms are the one which have the capacity to tolerate unfavorable conditions and these mechanisms are evolved for last few years.

Biosorption Mechanism: The structures of the microorganisms are very complex, which implies that there are many ways in which the microbial cell can take up the metal. The mechanisms for biosorption are different and can be categorized according to different criteria. Metal transport across cell membranes produces intracellular accumulation, which depends on metabolism of cell. This shows only feasible cells can be biosorbed. It is connected to an active microorganism defense system that responds in existence of toxic metal. During non-metabolic biosorption, metal digestion occurs through physicochemical interaction among metal and functional groups located on surface of microbial cells. This is based on the van der Waals forces adsorption, exchange of ions and chemical sorption that does not dependent on the cellular metabolism.

Response Surface Methodology (RSM): Box and colleagues created methodology for Response surface in 1950. This word originated from graphical perspective produced after mathematical model development, and its use was commonly embraced in texts of chemometrics. RSM includes a set of mathematical and statistical methods derived from suitability of an empirical model to the experimental information obtained in relation to experimental conception. To do this, use linear or quadratic polynomial function to describe the scheme under study and examine experimental conditions to optimize it.

2. MATERIAL AND METHODOLOGY

2.1. Seed Culture of Biofilm Producing Bacteria

Biofilm producing bacteria *Bacillus species* (TF_1) and *Staphylococcus aureus* (G_5) were inoculated into nutrient media at 37°C for 24 hour in the incubator. After 24 hour of inoculation those were used as a seed culture for the removing of chromium.

2.2. Selection of Carbon Source

Growth of microorganisms were influenced by glucose, sucrose, fructose, etc were the sources of carbon. Among these glucose and sucrose are most used carbon sources. These interns influence the biosorption process. Hence, selection of carbon source was carried out by using glucose and sucrose. The reduction of hexavalent chromium (Potassium dichromate used as a source of Cr (VI)) is carried out by adding 1% of glucose and sucrose in the nutrient media containing 500ppm of hexavalent chromium. The reduction was studied for every 24 hours of time intervals. Based on these results the carbon source was selected.

2.3. Viability Check of Bacteria in Wastewater

The wastewater from electroplating industry was collected from electroplating industry Belgaum. That wastewater was first sterilized at 15 lb pressure for 20 minutes in an autoclave and then it was allowed to cool. *Bacillus species* and *Staphylococcus aureus* cultures were inoculated in the wastewater and incubated for 24 hrs at 37°C. After completion of 24hrs of inoculation, the growth viability was checked by inoculating the wastewater in nutrient agar media by streak plate method. The growth was then observed.

2.4. Study of Enzymes by Cell Free Extract

2.4.1. Chromium Reducing Activity with Cell Free Extract

In nutrient media, bacterial cultures were grown with and without Cr (VI). Overnight grown culture were harvested separately by centrifugation at 10000 rpm for 5 mins, washed three times and re-suspended them in the phosphate buffer (pH 7). The cells are disrupted by centrifugation at 8000 rpm for 30 minute at 4°C and the supernatant is filtered by using 0.22μm filter paper. The sample was used for the chromate protein assay.

2.4.2. Protein Estimation by Lowry's Method

Total protein content of the sample was calculated by "Lowry's method" before and after the reduction of hexavalent chromium. About 1ml of sample was collected in the test tube and 5ml of alkaline copper sulphate was added to it. After the addition of alkaline copper sulphate it was incubated for 30 min. After 0.5 ml of Folin and Ciocalteus Phenol Reagent (FCR) was added and incubated again for 30 min in dark place. Then OD was taken at 660 nm. The protein concentration was calculated by plotting the graph OD v/s concentration of protein in the calibration curve.

2.4.3. Chromium Reducing Activities with Cell Free Assay

Overnight grown bacterial cells in the nutrient media with and without Cr (VI) are harvested by centrifugation at 3000 rpm for 10 mins separately; pellet was re-suspended with 10 m M Tris-HCl buffer (pH-7). Sample was boiled at 100°C for 5 min and centrifuged at 5000 rpm for 5 min. Then DPC Assay was carried out and OD was measured at 540 nm.

2.5. Response Surface Methodology (RSM)

To optimize the process, RSM technique was applied by using different factors i.e. Glucose, pH and biomass concentration. The RSM was designed by using Minitab software.

2.5.1. Influence of pH Reduction Efficiency

Nutrient broth containing 500 ppm of hexavalent chromium having different pH such as 4, 5, 6, 7 and 8 were inoculated with *Bacillus species* and *Staphylococcus aureus* seed inoculums (10% v/v) and at 37°C it was inoculated under agitation of 100 rpm. With 24 hrs of regular time interval the samples were withdrawn and the chromium concentration was analyzed by DPC assay method. Media without Cr (VI) served as control.

2.5.2. Influence of Glucose on Reduction Efficiency

Nutrient broth containing 500 ppm of hexavalent chromium having different glucose concentrations such as 0.2, 0.4, 0.6, 0.8 and 1 g was inoculated with *Bacillus species* and *Staphylococcus aureus* seed inoculums (10% v/v) and incubated at 37°C under agitation of 100 rpm. The samples were withdrawn at 24 hrs of intervening time and chromium concentration was analyzed by DPC assay method. Media without Cr (VI) served as control.

2.5.3. Influence of Biomass Concentration on Reduction Efficiency

Nutrient broth containing 500 ppm of hexavalent chromium having different biomass concentrations 2, 4, 6, 8 and 10 % v/v was inoculated with *Bacillus species* and *Staphylococcus aureus* seed inoculums and incubated at 37°C under agitation of 100 rpm. At 24 hrs of regular interval of time the samples were withdrawn and the chromium concentration was analyzed by DPC assay method. Media without Cr (VI) served as control.

2.6. Analytical Method for Hexavalent Chromium

The 1ml sample from the flasks is drawn at 24 hours of time intervals in pre-weighed eppendorf tubes and at 10000 rpm it was centrifuged for 5 min. The supernatant was used to examine the concentration of hexavalent chromium. The response with Diphenylcarbazide determined the concentration of Cr (VI) colorimetrically.

Diphenylcarbazide Assay: This method was carried out according to the protocol of [3][4]. About 100 µl of supernatant was collected in test tubes, 9.90 ml of distilled water was added to make the volume to 10 ml. Later, 2 ml of Diphenylcarbazide is added which reacts with 1–2 drops of conc. H_2SO_4 indicated by formation of pink color. The intensity of the color formation was greater for the sample with higher concentration of chromium which is measured colorimetrically at 540 nm. Later calculations were done to determine the chromium reduction. The graphs are plotted for OD v/s concentration.

$$\% \text{ of chromium reduction} = \frac{\text{initial concentration-final concentration}}{\text{Initial concentration}} \times 100$$

2.7. Reduction of Chromium in Wastewater

The reduction of hexavalent chromium in industrial wastewater was carried out by adding 10% inoculum. At 37°C the two isolates *Bacillus species* and *Staphylococcus aureus* were inoculated and incubated at 100rpm. The chromium concentration was analyzed by DPC method and also by atomic absorption spectrophotometer.

2.8. Scanning Electron Microscope (SEM)

SEM analysis for *Bacillus species* was done to check a morphological change that happens during reduction process. Hence, before and after reduction of Cr (VI), SEM images are taken and compared with its morphological changes.

3. RESULTS AND DISCUSSIONS

3.1. Selection of Carbon Source

After inoculation of *Bacillus species* and *Staphylococcus aureus* to the nutrient media containing 500ppm of Cr (VI) along with glucose (i.e. TF_1 (G) and G_5 (G)) and sucrose (i.e. TF_1 (S) and G_5 (S)), the sample was collected at 24 hours of time interval and analyzed for the reduction of Cr (VI) by DPC method.

Isolates are found to be reduced the Cr (VI) in both glucose and sucrose. But as we compare reduction in glucose and sucrose, reduction is more in glucose i.e. it shows 100% reduction within 10 days.

Table 4.1: Percentage Reduction of Cr (VI)

Sample	Number of Days									
	1	2	3	4	5	6	7	8	9	10
TF_1 (G)	-	16.19	54.28	69.52	75.23	81.08	89.90	94.23	98.64	100
TF_1 (S)	-	8.57	13.94	39.00	50.81	69.52	80.00	85.71	88.00	88.07

Table 4.1: Contd.,										
G_5 (G)	-	16.52	43.44	59.83	78.68	80.32	91.30	95.40	97.54	100
G_5 (S)	-	5.21	16.52	40.00	58.26	72.17	77.39	80.32	85.76	85.90

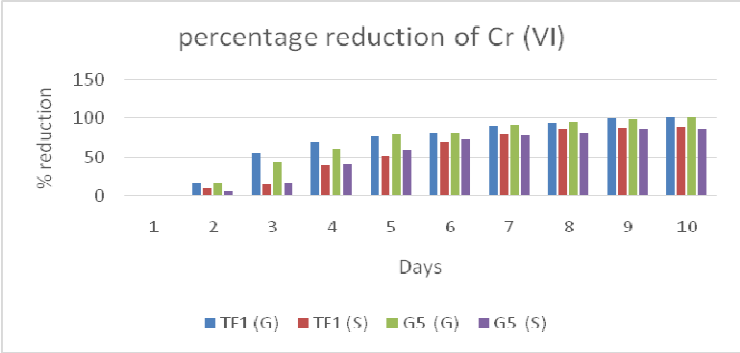

Figure 4.1: Percentage Reduction of Cr (VI).

In sucrose TF_1 (S) shows 88.07% and G_5 (S) shows 85.90% in 10 days. Based on this study, we came to know that using glucose as carbon source is more suitable than the sucrose. Hence in further process of reduction glucose is used as carbon source.

3.2. Initial Characteristics of Industrial Wastewater

The wastewater initial characteristics from electroplating industry are carried out. Those are

Table 4.2: Initial Characteristics of Electroplating Industrial Waste Water

Sl. No.	Parameters	Unit	Result
1	pH value	-	1.25
2	B.O.D (3 Days at 20˚C)	mg/L	2800
3	C.O.D	mg/L	9600
4	Total Organic Carbon (TOC)	mg/L	626.4
5	Electrical Conductivity	µs/cm	2,75,000
6	Chromium	mg/L	11.713
7	Colour	Hazen	<1

3.3. Viability of Bacteria in Industrial Waste Water

After 24 hours of inoculation the growth viability was checked by inoculating the wastewater in nutrient agar media by streak plate method. The growth was then observed.

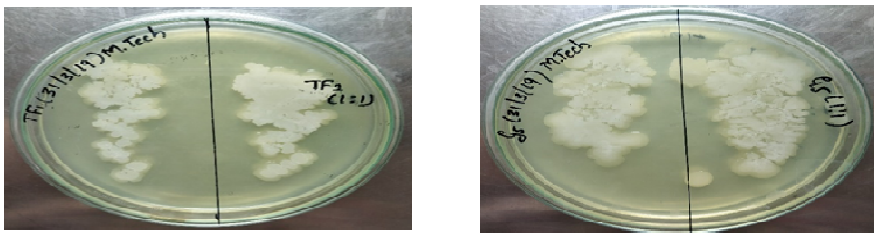

Figure 4.2: Viability of Isolates in Industrial Wastewater.

3.4. Enzymatic Study

3.4.1. Cell Free Extract

Cell free extract was carried out and protein was calculated by Lowry's method before and after the reduction of Cr (VI).

Table 4.3: Protein Concentration

Isolates	Before Reduction	After Reduction
Bacillus species	260 µg	470 µg
Staphylococcus aureus	270 µg	370 µg

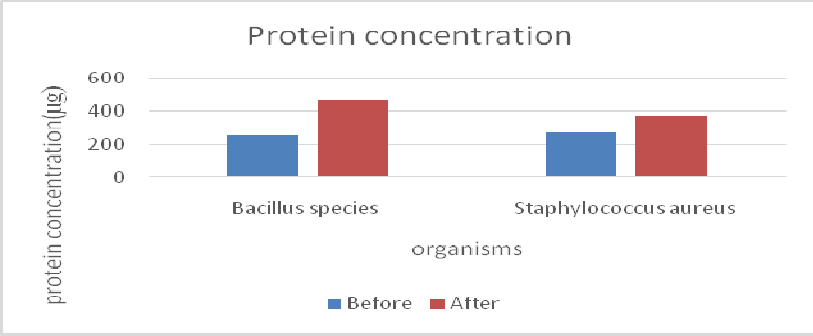

Figure 4.3: Protein Estimation.

Initially before reduction of Cr (VI), concentration of protein was less i.e. *Bacillus species* has 260 µg and *Staphylococcus aureus* has 270µg. After reduction of Cr (VI), concentration of protein was increased to *Bacillus species* TF_1 – 470 µg and *Staphylococcus aureus* – 370 µg. This suggests that as the concentration of protein increases the reduction of Cr (VI) also rises. Therefore, reduction of Cr (VI) is performed enzymatically.

3.4.2. Cell Free Assay

Table 4.4: Percentage Reduction of Cr (VI) by Cell Free Assay Method

Isolates	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day
Bacillus species	11.12	38.9	50	77.78	94.44	100
Staphylococcus aureus	15.38	23.07	61.53	84.61	92.3	97.14

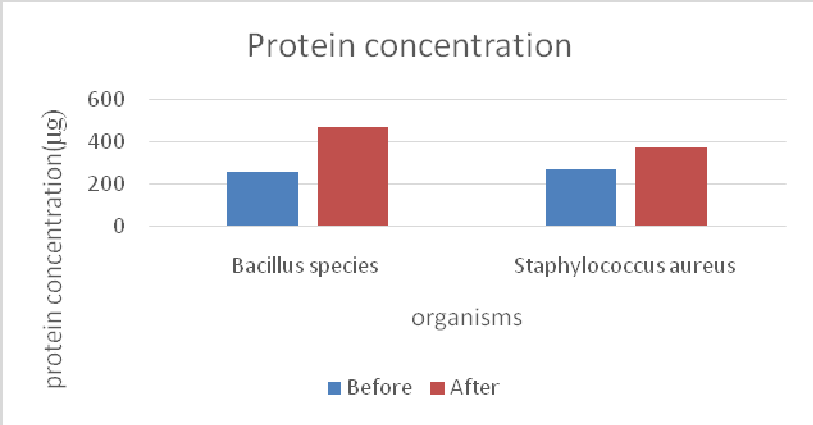

Figure 4.4: Cell Free Assay.

The experiment was carried out to check whether the enzymes were responsible for reduction of Cr (VI) or not. We have observed that the reduction takes place gradually which was detected by DPC test with a regular intervals. Chromium concentration was 90–99% at 0^{th} hr. The fast reduction was observed in *Bacillus species* as compared with *Staphylococcus aureus*. This indicates that protein concentration was maximum at *Bacillus species*, hence reduction was more.

3.5. Response Surface Methodology (Rsm) Studies

The 40 experiments were conducted to attain a quadratic model of biotransformation of Cr (VI) to Cr (III). Result of three independent variables that is pH, glucose and biomass concentration are indicated in the table besides with mean observed and predicted response. The following equation is derived from multiple regression analysis in order to explain the reduction of hexavalent chromium.

$$Y= -304.456+ 97.401 X A+ 108.982 X B+ 11.282 X C- 6.432 X A^2 – 135.067 X B^2 – 0.646 X C^2 +2.622 X AB – 1.425 X AC + 8.755 X BC.$$

Where Y is predicted reduction. Coded variables of A, B and C for pH, glucose and biomass concentration, respectively.

3.5.1. RSM for *Bacillus species*

Response Surface Regression: % Reduction Versus pH, Glucose, Biomass Concentration

Using coded units, analysis was carried out.

Calculated Valve for Regression Coefficients in % Reduction

Term	Coef	SE Coef	T	P
Constant	-304.456	34.0361	-8.945	0.000
pH	97.401	7.6257	12.773	0.000
Glucose	108.982	35.8794	3.037	0.005
Biomass Concentration	11.282	3.5879	3.144	0.004
pH*pH	-6.432	0.5364	-11.992	0.000
Glucose*Glucose	-135.067	13.4088	-10.073	0.000
Biomass Concentration* Biomass Concentration	-0.646	0.1341	-4.814	0.000
pH*Glucose	2.622	4.7542	0.551	0.585
pH*Biomass Concentration	-1.425	0.4754	-2.998	0.005
Glucose*Biomass Concentration	8.755	2.3771	3.683	0.001

S = 3.803 R-Sq = 95.5% R-Sq (adj) = 94.1%

Variance Analysis for % Reduction

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	9186.9	9186.9	1020.771	70.56	0.000
Linear	3	5909.1	2456.3	818.764	56.60	0.000
Square	3	2947.2	2947.2	982.412	67.91	0.000
Interaction	3	330.6	330.6	110.209	7.62	0.001
Residual Error	30	434.0	434.0	14.466		
Lack-of-Fit	5	235.2	235.2	47.048	5.92	0.001
Pure Error	25	198.7	198.7	7.949		
Total	39	9620.9				

R indicates an observation with a large standardized residual.

Table 4.5: Analysis of RSM by TF_1 Bacteria

Run order	pH	Glucose	Biomass Concentration	% of Reduction	Predicted
1	7	0.8	8	100	102.764
2	6	0.6	6	100	99.261
3	6	0.6	6	100	99.261
4	6	0.6	6	100	99.261
5	7	0.4	4	97.91	100.404
6	5	0.4	8	65	68.921

Table 4.5: Contd.,					
7	6	0.6	2	100	87.985
8	5	0.4	4	70	69.276
9	5	0.8	8	79	80.941
10	7	0.4	8	90	88.647
11	6	0.6	6	100	99.261
12	6	0.6	2	82	87.985
13	6	0.6	6	100	99.261
14	4	0.6	6	52	47.057
15	6	0.6	6	100	99.261
16	6	0.2	6	75	71.586
17	6	0.6	10	91.3	89.881
18	5	0.8	8	79	80.941
19	4	0.6	6	51	47.057
20	5	0.4	8	65	68.921
21	8	0.6	6	100	100.008
22	6	0.6	10	91.3	89.881
23	6	1.0	6	88	83.715
24	7	0.8	4	100	100.513
25	5	0.4	4	65.21	69.276
26	6	0.6	6	100	99.261
27	5	0.8	4	64	67.288
28	7	0.8	8	100	102.764
29	7	0.4	8	90	88.647
30	7	0.8	4	100	100.513
31	6	0.2	6	70.47	71.586
32	6	1.0	6	86	83.715
33	6	0.6	6	100	99.261
34	8	0.6	6	100	100.008
35	5	0.8	4	59	67.288
36	6	0.6	6	100	99.261
37	6	0.6	6	100	99.261
38	6	0.6	6	100	99.261
39	6	0.6	6	100	99.261
40	7	0.4	4	97.91	100.404

Polynomial equation is prepared by using coefficients

$Y= a_0 + a_1 A+ a_2 B + a_3 C + a_4 A^2 + a_5 B^2 + a_6 C^2 + a_7 AB + a_8 AC + a_9 BC$

$Y= -304.456+ 97.401$ X pH+ 108.982 X glucose+ 11.282 X biomass- 6.432 X pH^2 – 135.067 X $glucose^2$ – 0.646 X biomass $concentration^2$ +2.622 X pH x glucose – 1.425 X pH x biomass concentration + 8.755 X glucose x biomass concentration.

T and P are the results of regression got from CCD along with coefficients and constants. T value helps to determine the importance of regression coefficients of different parameters and value of P shows the least level of significance which leads to rejection of the null hypothesis. R^2 value should be high. R^2 obtained here is 95.5% and the predicted value is 94.1 %. This indicates the elevated reliance and also correlation between the expected value and the acquired value.

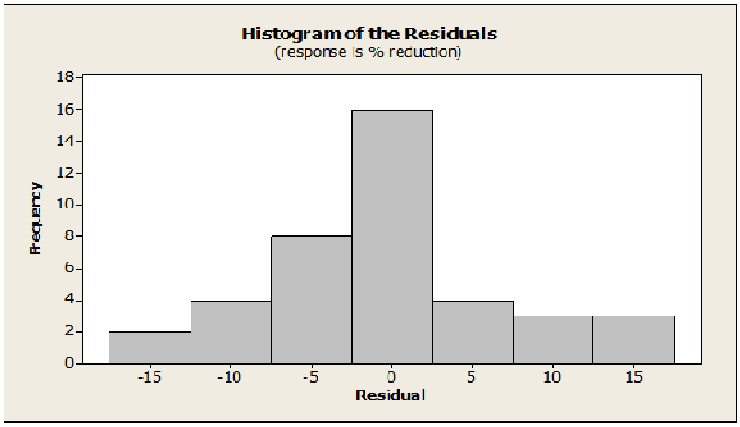

Figure 4.5: Histogram of Residuals.

Histogram indicates the distribution of residuals where the central point is found to be more important. In this, the centre points are pH 6, glucose 0.6 mg/l and 6% biomass concentration, which indicates the optimum parameters for reduction of Cr (VI).

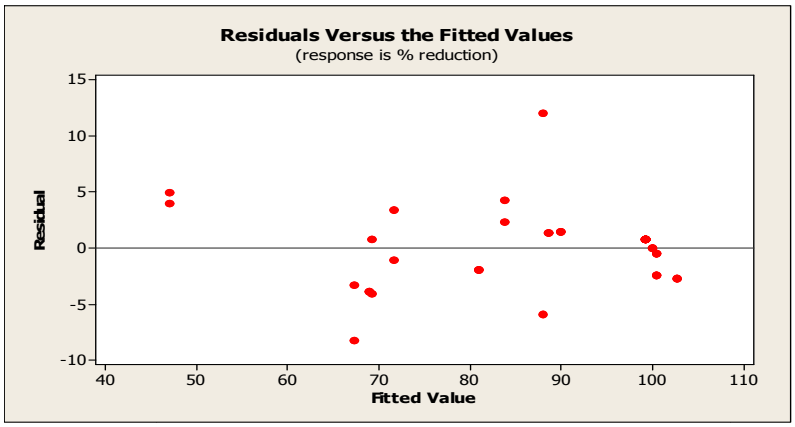

Figure 4.6: Residuals V/S Fitted Values.

This graph explains about the predicted response. Here the residuals are found spread around zero which shows that errors have a constant variance.

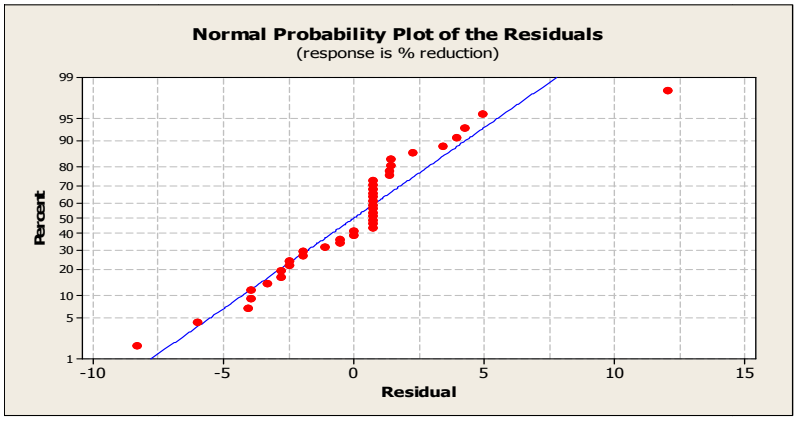

Figure 4.7: Probability Plot of Residuals.

The plot in the graph below shows the normal distribution as many points align on the straight line. The presence of the residual values at the time of regression. Here the plot points should be near the straight line indicating the normal distribution.

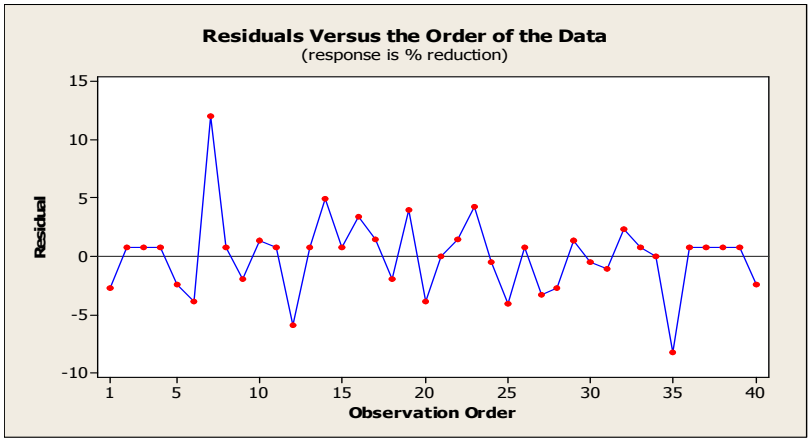

Figure 4.8: Residuals v/s Order of the Data.

This is a graph which shows the normal distribution as many points align on the straight line. The presence of the residual values at the time of regression. In this, the points should be near the straight line indicating the normal distribution. The residual plots are scattered in the range of −10 to +10 and one has gone beyond +10.

[5] Narasimhulu K (2017) also conducted the batch experiments to optimize the biosorption process by *Bacillus subtilis*, they got optimum values at pH 4.0, concentration of biomass is 2.0 mg/ml, temperature of 32°C and contact time of 30 min and the percent biosorption of Cr (VI) was found to be 80%. The present study using *Bacillus species* got optimum values at pH 6, glucose 0.6 mg/l and 6% biomass concentration.

3.5.2. RSM for *Staphylococcus aureus*

The following equation is derived from multiple regression analysis in order to explain the reduction of hexavalent chromium.

$$Y= 286.394 – 55.637 X A – 233.743 X B - 13.285 X C + 5.303 X A^2 + 86.621 X B^2 + 0.659 X C^2 + 11.478 X AB – 0.097 X AC + 9.902 X BC.$$

where Y is predicted reduction coded variables, A, B and C are the for pH, glucose and biomass concentration, respectively.

RSM: % reduction versus pH, glucose conc, biomass conc

Coding units were used for the analysis.

Calculated Regression Coefficients for % Reduction

Term	Coef	SE Coef	T	P
Constant	286.394	39.9425	7.170	0.000
pH	-55.637	8.9490	-6.217	0.000
Glucose Concentration	-233.743	42.1057	-5.551	0.000
Biomass Concentration	-13.285	4.2106	-3.155	0.004
pH*pH	5.303	0.6294	8.425	0.000
Glucose Concentration* Glucose Concentration	86.621	15.7356	5.505	0.000
Biomass Concentration* Biomass Concentration	0.659	0.1574	4.187	0.000
pH*Glucose Concentration	11.478	5.5793	2.057	0.048
pH*Biomass Concentration	-0.097	0.5579	-0.173	0.864
Glucose Concentration* Biomass Concentration	9.902	2.7896	3.549	0.001

S = 4.463 R-Sq = 93.5% R-Sq(adj) = 91.6%

Variance Analysis for % Reduction

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	8652.29	8652.29	961.366	48.26	0.000
Linear	3	6552.11	1015.90	338.632	17.00	0.000
Square	3	1764.28	1764.28	588.094	29.52	0.000
Interaction	3	335.90	335.90	111.967	5.62	0.004
Residual Error	30	597.66	597.66	19.922		
Lack-of-Fit	5	507.70	507.70	101.539	28.22	0.000
Pure Error	25	89.96	89.96	3.598		
Total	39	9249.95				

Table 4.6: Analysis of RSM by G_5 Bacteria

Run Order	pH	Glucose	Biomass Concentration	% of Reduction	Predicted
1	7	0.8	8	90	83.35
2	6	0.6	6	50	51.916
3	6	0.6	6	50	51.916
4	6	0.6	6	50	51.916
5	7	0.4	4	85.4	79.821
6	5	0.4	8	50.78	47.811
7	6	0.6	2	60.63	62.526
8	5	0.4	4	56.50	55.414
9	5	0.8	8	52.45	50.533
10	7	0.4	8	77	71.446
11	6	0.6	6	58	51.916
12	6	0.6	2	57.69	62.526
13	6	0.6	6	50	51.916
14	4	0.6	6	44.26	44.516
15	6	0.6	6	50	51.916
16	6	0.2	6	62.54	66.383
17	6	0.6	10	58.26	62.390
18	5	0.8	8	52.45	50.533
19	4	0.6	6	43.26	44.516
20	5	0.4	8	50.78	47.811
21	8	0.6	6	95	101.740
22	6	0.6	10	58.26	62.390
23	6	1.0	6	62.96	65.167
24	7	0.8	4	78.82	75.883
25	5	0.4	4	55.52	55.414
26	6	0.6	6	50	51.916
27	5	0.8	4	45	42.294
28	7	0.8	8	90	83.350
29	7	0.4	8	73.83	71.446
30	7	0.8	4	82	75.883
31	6	0.2	6	64.61	66.383
32	6	1.0	6	58	65.167
33	6	0.6	6	50	51.916
34	8	0.6	6	95	101.740
35	5	0.8	4	46.64	42.294
36	6	0.6	6	50	51.916
37	6	0.6	6	50	51.916
38	6	0.6	6	50	51.916
39	6	0.6	6	50	51.916
40	7	0.4	4	85.4	79.821

Polynomial equation is prepared by using coefficients

$Y= a_0 + a_1 A+ a_2 B + a_3 C + a_4 A^2 + a_5 B^2 + a_6 C^2 + a_7 AB + a_8 AC + a_9 BC$

$Y= 286.394 – 55.637 X pH – 233.743 X glucose - 13.285 X biomass + 5.303 X pH^2 + 86.621 X glucose^2 + 0.659 X biomass concentration^2 +11.478 X pH x glucose – 0.097 X pH x biomass concentration + 9.902 X glucose x biomass concentration.$

T and P are the results of regression got from CCD along with coefficients and constants. T value helps to determine the importance of regression coefficients of different parameters and P value show least level of significance which leads to rejection of null hypothesis.

R^2 value should be high. R^2 obtained here is 93.5% and the predicted value is 91.6 %. This indicates the high dependence and correlation between predicted and the obtained value.

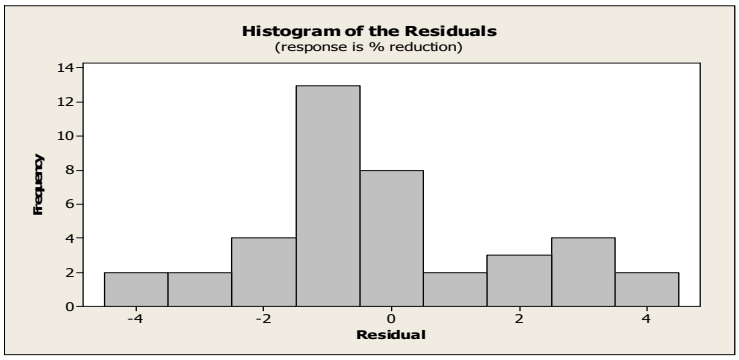

Figure 4.9: Histogram of Residuals.

Histogram indicates the residuals distribution where the central point is found to be more important. In this, the optimum points lie between 0 and −2, this indicates the optimum value of pH is 5–6, glucose 0.4–0.6 mg/l and 4-6% biomass concentration. But it is near to the zero. Hence here we are considering the optimum parameters at pH 6, glucose 0.6 mg/l and 6% biomass concentration, which indicates the optimum parameters for reduction of Cr (VI).

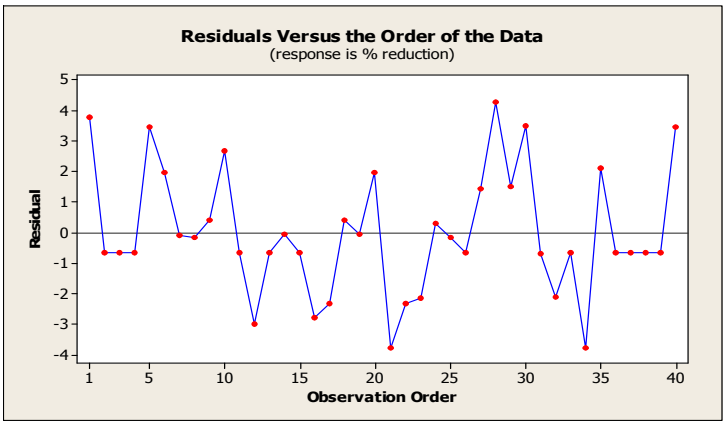

Figure 4.10: Residuals v/s Order of the Data.

This is a graph which shows the normal distribution as many points align on the straight line. The presence of the residual values at the time of regression. In this the points should be near the straight line indicating the normal distribution. The residual plots are scattered in the range of −4 to +4 and one has gone beyond +4.

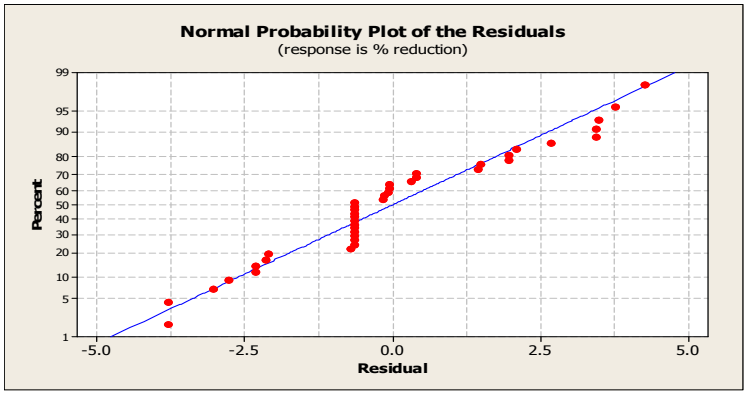

Figure 4.11: Normal Probability Plot.

The plot in the graph below shows the normal distribution as many points align on the straight line. The presence of the residual values at the time of regression. Here in the graph, points should be near the straight line indicating the normal distribution.

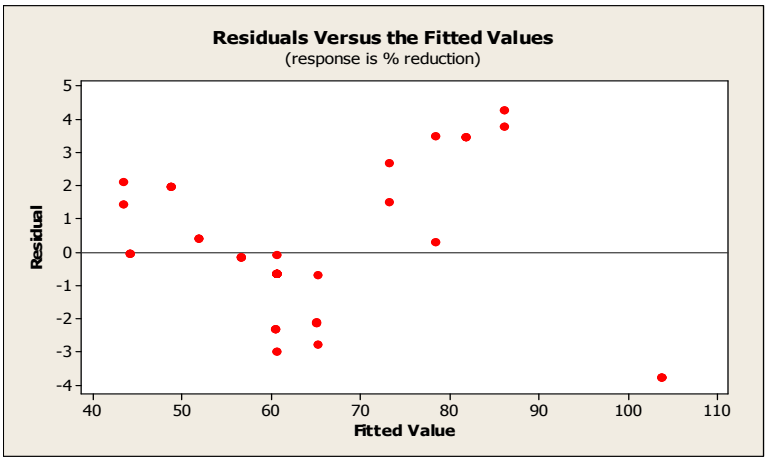

Figure 4.12: Residuals v/s Fitted Values.

This graph explains about the predicted response. Here the residuals are found spread around zero which shows that errors have a constant variance.

[6]Z. A. Zakaria (2007) studied RSM by *S. aureus*, they got optimum parameters at pH 7 and initial chromium concentration at 20–400 mg/l. But in our studies the optimum pH is 6 at 500 ppm of chromium concentration.

3.6. Reduction of Cr (VI) in Wastewater

After 7 days of inoculation, the wastewater is analyzed for the reduction of Cr (VI) by atomic absorption spectrophotometer. Both isolates reduced Cr (VI) 100%. Initial concentration was 11.713mg/L. After reduction it is reduced to 0.0 mg/L. This indicates that both the isolates are capable of reducing the Cr (VI) in electroplating industrial wastewater.

Table 4.7: Characteristics of Wastewater after Treatment by *Bacillus Species*

Sl. No	Parameters	Units	Results
1	COD	mg/L	6160
2	BOD (3 days at 20˚C)	mg/L	2814
3	Cr (VI)	mg/L	< 0.05

Table 4.8: Characteristics of Wastewater after Treatment by *Staphylococcus aureus*

Sl. No	Parameters	Units	Results
1	COD	mg/L	6480
2	BOD (3 days at 20^{o}C)	mg/L	2898
3	Cr (VI)	mg/L	< 0.05

3.7. SEM- Image of *Bacillus Species*

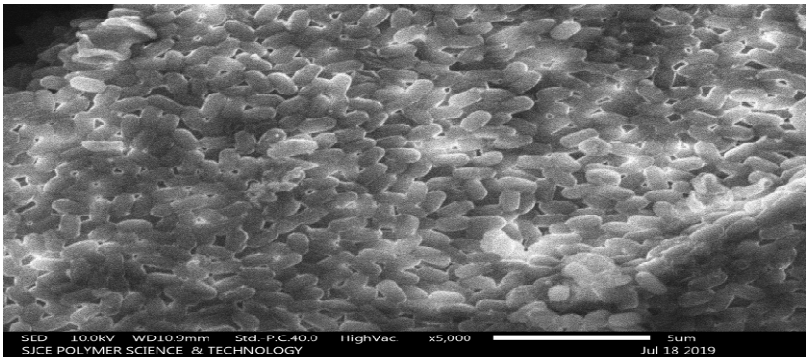

Figure 4.13: SEM Image of Biosorbent before Biosorption.

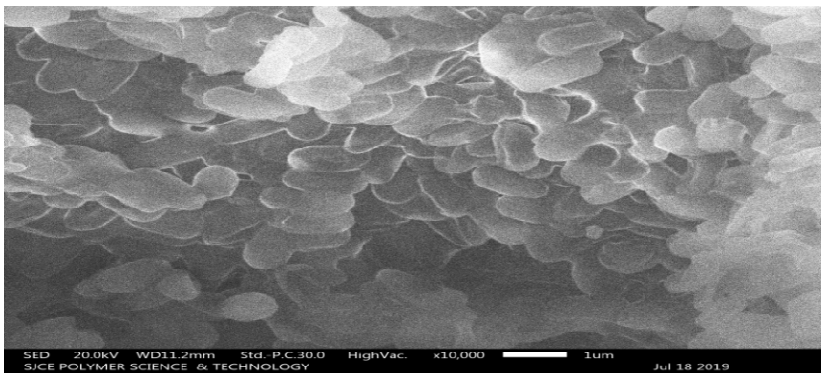

Figure 4.14: SEM Image of Biosorbent after Biosorption.

The SEM micrographs of *Bacillus species* before and after Cr (VI) reduction was shown in figures 4.13 and 4.14, respectively. Comparing these two figures it is noticeable that, morphology of sorbent surface changes during adsorption process. This may be due to adhesion or reduction of Cr (VI) by the isolate.

4. CONCLUSIONS

The electroplating industrial wastewater from Belgaum was collected and its initial characters were analyzed. It contains 11.713 mg/L of chromium. Hence, to remove this Cr (VI) two isolates *Bacillus species* and *Staphylococcus aureus* were used as biosorbents. The biosorption technique was carried out for Cr (VI) removal. Designs of experiments were conducted by using RSM to optimize the Cr (VI) reduction process by using Mini Tab software. From this, we came to know that the Cr (VI) was reduced maximum at pH 6, glucose concentration at 0.6 mg/L and biomass concentration at 6%. As we compare the two isolates, *Bacillus species* has shown more efficient reduction of Cr (VI) than *Staphylococcus aureus*. Study of enzymes was also carried out. Study confirms that the reduction of Cr (VI) was takes place enzymatically. As the concentration of protein increases the reduction of Cr (VI) also increased. This indicates that reduction is based on

enzymatic pathway. The isolates were inoculated into wastewater to remove Cr (VI). The both *Bacillus species* and *Staphylococcus aureus* isolates were removed 100% hexavalent chromium within 4 days in the electroplating industrial wastewater. Even SEM analysis for *Bacillus species* was carried out before and after reduction to check morphological changes in isolate and the changes are observed.

REFERENCES

1. *Al-Asheh, S," Predictions of Binary Sorption Isotherms for the Sorption of Heavy Metals by Pine Bark using Single Isotherm Data", Chemosphere, 2000, Vol. 41 (5), pp 659–665.*
2. *Muhammad, S., M. T. Shah, and S. Khan, "Health Risk Assessment of Heavy Metals and their Wrce apportionment in Drinking Water of Kohistan region, Northern Pakistan". Microchemical Journal, 2011, Vol. 98 (2), pp 334–343.*
3. *Greenberg, A. E., Trussell, R. R. and Clesceri, L. S. (1992). "Standard Methods for the Examination of Water and Wastewater", 18th ed., American Public Health Association, New York.*
4. *Turick, C. E. and Apel, W. W. (1997). Method for in situ or ex situ bioremediation of hexavalent chromium contaminated soils and/or groundwater. US Patent 5681739.*
5. *Narasimhulu K, "Optimization of Batch Biosorption of Cr(VI) and Cu(II) Ions from Wastewater using Bacillus subtilis", Sci. Fed Materials Research Letters, 2017, Vol. 1 (3), pp 1–13.*
6. *Zakaria Z. A, Aruleswaran N, Kaur S and W. A. Ahmad, "Biosorption and Bioreduction of Cr(VI) by Locally Isolated Cr-Resistant Bacteria", Water Science & Technology, 2007, Vol. 56 (8), pp 117–123.*

AUTHORS PROFILE

Smt. MADHUMALA Y: Asst. Professor in Basaveshwar Engineering College (BEC), Bagalkot, Karnataka. Administration Responsibilities: (a) At University level (b) at College level (c) Others, in the following format worked as BOS Member, Bt zillion coordinator MIS-TEQIP (Dept coordinator) Valuation Coordinator, Project Coordinator, Proof Reading.

Educational qualification: She has completed M Sc. through Karnatak University Dharwad in 2002 and M. Phil through Vinayak Missions University Salem in 2008 and registered Ph. D through BEC, Bagalkot (VTU, Belgaum university), with research area in Biofilm induced reduction of Hexavalent chromium by indigenous microbes. Grants were received for the four projects, in that two projects were funded by KSCST and other two were by TEQIP. Published two journal papers, one is in International Journal of Advanced Research Methodology in Engineering and technology and another in IJTIMES. Presented a paper in national conference on cyanide remediation using microbes using adsorption and biosorption techniques. Expert on areas like bioremediation, waste management, microbiology.

MANJULA MATHAPATI: Completed Master of Technology in Environmental Engineering with CGPA 9.48 in Aug 2019 through Basaveshwar Engineering College (BEC), Bagalkot, Karnataka. Carried out project work and title is "Removal of heavy metal from industrial wastewater using biofilm producing bacteria", which is funded by TEQUIP. In 2017 completed Bachelor of Engineering in Biotechnology with CGPA 8.93 through BEC Bagalkot, Karnataka. Research is done in the area of bioremediation with the title "Biofilm production and reduction of Hexavalent Chromium in Leachate". Awarded first prize with gold medal in both BE and M.Tech. Participated in State level science exhibition conducted in the year 2017.

Dr. VEENA S. SORAGANVI: Professor in Civil Engineering Department, Basaveshwar Engineering College (BEC), Bagalkot, Karnataka. Educational qualification is, completed B.E in Karnatak University Dharwad/B.E.C. Bagalkot. M.Tech is through IIT Kanpur, Uttar Pradesh and also completed PhD in Indian Institute of Science Bangalore, Karnataka in 2005. The title of the PhD topic is "Numerical and Stochastic Modeling of Flow and Transport in the Vadose Zone". In 2009, Post-Doctoral Research was carried out with the title "Groundwater Flow and Contaminant Migration" through Institut de Mécanique des Fluides de Toulouse, France, Unité Mixte de Recherche CNRS-INPT-UPS) at Toulouse, France. Published 14 International journal papers and also published one in National journal paper attended and presented papers in 13 international conferences 3 national conference. Expert in areas of hydraulics, GIS, waste management.